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STRUCTURAL AND FUNCTIONAL CONNECTIVITY IN MULTIPLE SCLEROSIS

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Título

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Abstract

Introduction: Multiple Sclerosis (MS) is an inflammatory, demyelinating disease of the central nervous system with diffuse damage in white and gray matter. Recently, it has been documented that the study of functional connectivity in the human brain at rest is an important tool to assess brain regional interactions during rest or task-performance. Currently, studies on the functional connectivity of the resting state networks in MS present contradictory results.

Objectives: We will analyze the functional connectivity at a whole brain network level and in the Default Mode Network (DMN), Dorsal Attention Network (DAN) and Ventral Attention Network (VAN). Additionally, we will analyze the association between the functional connectivity of these resting state networks, disease duration and cognitive efficiency in the MS group.

Methodology: We compared a group with MS diagnosis (N=23) with a healthy group (N=21), matched in age and gender, using a resting-state functional magnetic resonance imaging acquisition. The following connectivity measures were analyzed: a) intra-network changes in the functional and structural connectivity of the DMN, DAN and VAN; b) changes in whole-brain sub-networks and; c) correlation between functional connectivity of the DMN, DAN and VAN, disease duration and cognitive efficiency composite in the clinical group.

Results: Data shows that MS group displayed decreased functional connectivity in several paired regions of the DMN, DAN and VAN that was consistent with our expanded analysis performed at a whole brain functional connectome. Additionally, in the MS group, increased functional connectivity in pairs of the DMN, DAN and VAN was associated with disease duration and cognitive efficiency.

Conclusion: This regional and whole brain level approach is useful to study MS and can provide new insights regarding the brain functional reorganization and compensatory mechanisms that occur within the course of the disease.

Keywords: Multiple Sclerosis, Functional And Structural Connectivity, Resting State Networks

Resumo

Introdução: A Esclerose Múltipla (EM) é uma doença inflamatória, desmielinizante do sistema nervoso central, caracterizada por uma lesão difusa da substância branca e substância cinzenta. Recentemente, foi documentado que o estudo da conectividade funcional do cérebro em repouso é uma ferramenta importante para estudar interações cerebrais regionais que correm durante o repouso ou na execução de uma tarefa.

Objectivos: Será analisada a conectividade cerebral a um nível cerebral global e regional; especificamente nas redes de repouso *Default Mode Network* (DMN), Rede Atencional Dorsal e Rede Atencional Ventral. Adicionalmente, será analisada a associação entre a conectividade funcional das redes cerebrais de repouso, a duração da doença e a eficiência cognitiva no grupo com EM.

Metodologia: Foi comparado um grupo com diagnóstico de EM (N=23) com um grupo controlo saudável (N=21), emparelhado em sexo e idade, usando uma aquisição de ressonância magnética funcional. As seguintes medidas de conectividade foram analisadas: a) mudanças de conectividade funcional e estrutural intra-rede na DMN, Rede Atencional Dorsal e Rede Atencional Ventral; b) mudanças de conectividade funcional nas redes cerebrais globais e ; c) correlação entre a conectividade funcional da DMN, Rede Atencional Dorsal e Rede Atencional Ventral, duração da doença e compósito de eficiência cognitiva no grupo clínico.

Resultados: Os dados evidenciaram que o grupo com EM apresentava uma diminuição da conectividade funcional em vários pares de regiões da DMN**,** Rede Atencional Dorsal e Rede Atencional Ventral, o que foi consistente com a análise mais expandida realizada ao nível do conectoma cerebral. Adicionalmente, no grupo com MS, um aumento da conectividade funcional estava associado a maior duração da doença e melhor eficiência cognitiva.

Conclusão: O estudo da conectividade funcional a um nível cerebral global e regional revela-se de utilidade para estudar a EM e pode fornecer novas formas de conceptualizar a reorganização cerebral funcional e mecanismos compensatórios que ocorrem no curso da doença.

Palavras Chave: Esclerose Múltipla, Conectividade Estrutural e Funcional, Redes Cerebrais de Repouso.

Introduction

Multiple Sclerosis (MS) is an inflammatory, demyelinating disease of the central nervous system that affects brain parenchyma. Approximately 2 million people have the MS diagnosis worldwide [1], occurs mainly in adulthood with a median estimated female/male ratio of 2:1 [2,3], and is associated with an increased society burden, as in most cases is associated with chronic disability. Clinically, MS is characterized by a high variable and unpredictable clinical presentation, and is classified according to four major categories regarding the course of the disease: Relapsing-Remitting MS (RRMS), Primary-Progressive MS, Secondary-Progressive MS (SPMS) and Progressive-Relapsing MS [4]. Several mechanisms underlying the main inflammatory, demyelinating and neurodegenerative processes have been documented, including the role of adhesion-molecule biology, axonal dysfunction and channelopathy, terminal-injury effector mechanisms, among others, but no single comprehensive theory has emerged until now – see [5] for a review. The role of cytokines, nitric oxid, proteases, superoxides and oxidative stress, CD8+T Cells, Th-17 cells, B cells, major histocompatibility complex, Epstein-Barr Virus infection, lack of vitamin D, and glutamate excitotoxicity has been involved in the demyelinating process and pathophysiology of MS [5-7]. This axonal involvement also extends to the neuron cell body, which is evident in Magnetic Resonance Imaging (MRI) as white matter and gray matter alterations, respectively. In fact, diffuse damage in white and gray matter is known to occur in MS, with cortical lesions and gray matter atrophy are likely to existing even before the appearance of white matter lesions [8-10].

Conventional and advanced quantitative and functional MR techniques are widely established clinical tools [11] and have been progressively improving our knowledge regarding the diagnosis and evolution of MS, as well as providing important information regarding its pathophysiology [12] and the role of white and gray matter alterations [13].

Recently, it has been documented that the study of functional connectivity in the human brain at rest is being widely used to assess brain regional interactions that comprise the resting state networks (RSNs) [14,15], both during resting periods and task-induced deactivations. Importantly, alterations in the patterns of RSNs have been associated with several disease states and neuropsychiatric disorders [16-19].

The most studied RSNs is the Default Mode Network (DMN), which is characterized by a network of brain areas that present high metabolic activity when the brain is “at rest” and the individual is not focused on any external demand. This network displays a high degree of functional connectivity between various interacting brain areas. Typically, the DMN comprises areas of the posterior cingulate cortex and adjacent precuneus, the medial

prefrontal cortex, the medial, lateral and inferior parietal cortex, and the medial and inferior temporal cortex [20,21]. The DMN is thought to serve important cognitive functions as intrinsic attention, self-referential processing, autobiographical and prospective memory [19,22,23]. It is also considered a task-negative network [24], as task-induced deactivations of the DMN have been functionally associated with a wide range of goal-directed tasks [25] and increased cognitive performance [26,27], supporting the role of this RSNs in maintaining a normal cognitive level. While the DMN shows deactivation during cognitively demanding tasks [26,28], functional connectivity of two other largely segregated canonical RSNs – a bilateral dorsal attention network (DAN, including the dorsal frontal and parietal cortices) and a ventral attention network (VAN, largely right-lateralized and which includes the ventral frontal and parietal cortices, the insular cortex and subcortical regions) typically increases during task performance, particularly in attention-demanding tasks, and accordingly, are often referred as task-positive networks. While the DAN has been associated with goal-directed, top-down attention processes as inhibitory control, working memory and response selection, the VAN is related with salience processing and mediates stimulus-driven, bottom-up attention processes [29-31].

Importantly, in MS an increased synchronization of the DMN, executive function, attention system, bilateral frontoparietal (DAN and VAN) and sensoriomotor networks was observed in patients with symptoms suggestive of multiple sclerosis (clinically isolated syndrome) when compared with patients with RRMS and healthy controls [32]. Nevertheless, no RSNs functional connectivity differences were found in patients with RRMS in comparison to controls, despite gray matter atrophy and changes in white matter diffusivity measures were observed between these groups. The authors suggested that this altered connectivity pattern possibly reflects an early cortical cerebral reorganization process in clinically isolated syndrome. This hypothesis was further corroborated by evidence derived from others [33], but whether this enhanced connectivity is linked to cognition is still an unsolved issue.

While this increased functional connectivity of the RSNs was observed in early MS, studies addressing the intrinsic brain connectivity in patients with chronic secondary progressive and primary progressive MS, showed a decreased functional connectivity of the RSNs in several brain regions of the DMN such as the left precentral gyrus, anterior cingulate cortex, and medial prefrontal cortex, when compared to controls [34]. These RSNs functional connectivity abnormalities were clinically significant, as they were associated with cognitive impairment and physical disability [34-38], with the more discriminative changes being observed in the fronto-parieto-temporal regions involving the DMN, DAN and VAN. [39]. In this line, a decreased functional connectivity of the anterior cingulate cortex was associated with the level of cognitive impairment (more pronounced in more cognitively impaired patients) in

patients with MS [34,40]. Additionally, an anatomofunctional study of the DMN brain regions showed that the functional connectivity is mostly altered between the medial prefrontal cortex and the posterior cingulate cortex in patients with MS with cognitive impairment [41]. Moreover, this abnormal RSNs functional connectivity in MS is consistent with altered white matter integrity measures, which have been documented in commissural, association and projection tracts [42-45]. Together, this evidence suggests a disruption of the global functional brain network organization in MS, which was corroborated by a recent study that applied graph analysis to RSNs in MS [46].

Overall, the results from studies assessing functional connectivity of the RSNs in MS present mixed results, an the functional connectivity of the RSNs analysis at regional and whole brain levels and in relation with cognitive functioning is still not addressed. Therefore, taking into account evidence that the functional connectivity of the fronto-parietal-temporal regions is more affected by the disorder, we will assess brain the DAN, VAN and DMN functional connectivity. For that, we will compare a group with MS with a healthy group taking into account the following connectivity measures: a) intra-network changes in the functional and structural connectivity of the DMN, DAN and VAN and; b) changes in whole-brain sub-networks associated with MS. Additionally and considering that there is an association between cortical lesion load, functional and structural connectivity and both physical and cognitive disability in MS [9,32,47,48], we will analyze the association between the functional connectivity of the DMN, DAN and VAN, disease duration and cognitive efficiency in the MS group.

Materials and Methods

Participants

A group of 23 participants with clinically definitive MS diagnosis [9 males, mean age (±SD) was 38.17 (±9.42), ranging from 20-55 years] was recruited from the database of the Neurology Department of a General Hospital [Hospital Geral de Coimbra (Covões) – Centro Hospitalar de Coimbra] and referred by the neurologists associated with the project. The following patient eligibility criteria was employed a) definite MS diagnosis [11]; b) stable disease on the 3 months prior to the study; c) RRMS or SPMS course of disease; d) neurological disability level (EDSS) less than 5; and e) treatment with imunomodulatory medication. Relative to the course of disease, 21 patients presented a RRMS and 2 presented a secondary progressive course of disease. The mean disability level as assessed by the EDSS had a median of 1.5 (ranging from 0.0 to 4.5). Mean time from diagnosis was

36.74 (±57.26) months, ranging from 1 to 228 months and total white matter lesion volume (M, SD) was 4807.23 (7440.71) mL.

To set a comparison with the clinical sample, a group of 21 healthy adults (HC) with similar distribution of age [M=35.95 (±7.27)] and gender proportion, was recruited from the community. Excluding criteria for both groups included a) the presence of any medical condition that could influence cognition (CNS disorder); b) history of brain injury; c) current or past psychiatric disorder; d) psychoactive substances abuse (current or past); e) severe visual disturbances; and f) attacks on the previous month (MS group).

All participants and patients were informed about the research study and gave written informed consent. Approval for the project was obtained from the Ethical Committee [Hospital Geral de Coimbra (Covões) – Centro Hospitalar de Coimbra] and from the Ethical Board of the School of Psychology (University of Minho). All participants and patients were right-handed except for two healthy participants (one was left-handed and another one was ambidextrous). Differences between groups in terms of demographical and cognitive variables are displayed in Table 1.

Neuropsychological assessment

The neuropsychological assessment protocol included the MACFIMS battery [49]. This battery resulted from a consensus of experts in the field and covers the assessment of the cognitive domains typically affected in MS, namely processing speed, working memory, memory, executive functioning and verbal fluency [49]. Nevertheless, some authors suggest that processing speed deficit is the core cognitive deficit of MS and the Symbol Digit Modalities Test (SDMT) is the best available measure to detect it [50-52]. Moreover, this test has demonstrated sensitivity to brain insult for which is considered a useful screening measure for brain damage and cerebral dysfunction in both children and adults [53]. Additionally, the Paced Auditory Serial Addition Test (PASAT) was developed to monitor cognitive impairment after concussion. PASAT is an auditory task that requires fast information processing, allocation of attention, working memory and calculation ability [49]. Both measures (SDMT and PASAT) are sensitive to subtle deficits in MS, show good psychometric properties [49] and have been associated with neuroimaging findings [54,55]. Therefore, and considering our focus in the attentional networks (DAN and VAN) we created a cognitive efficiency composite based on these two measures in order to correlate them with our neuroimaging outcomes. This cognitive efficiency composite was calculated as the average of the *z*-scores in SDMT and PASAT, based on the performance of a larger group of healthy adults, as described elsewhere [56].

Imaging acquisition

Magnetic Resonance (MR) images were obtained in a clinical approved 3T MRI (Siemens Trio Tim). Structural and functional sequence parameters of MRI acquisition were as follows. The T1-weighted 3D volumetric acquisition was obtained with a 3D MPRAGE (Magnetization Prepared Rapid Gradient Echo) sequence performed with the following protocol: time of repetition (TR)/ time of inversion (TI)/ time of echo (TE) = 2300 ms/900 ms/2,98 ms/, flip angle (FA)= 9º, field of view (FoV)=256 mm2, 160 sagittal slices and isotropic voxel size = 1x1x1mm^3. MPRAGE images were used as auxiliary for the spatial normalization of the functional imaging data. For the functional acquisition, a 2D echo planar imaging (EPI) blood-oxygen-level dependent (BOLD) sensitive sequence with the following parameters was used: TR/TE = 2000ms/30ms, FA=90º, FOV=240 mm2, voxel size=3x3x3 mm^3, 46 axial slices with no slice gap and 150 volumes were collected while patients were instructed to keep with their eyes closed, not to fall asleep and trying not to think of anything in particular. The same MR protocol was followed for every patient and healthy participant. The diffusion weighted acquisition was performed with an EPI sequence with the following parameters: TR/TE=8000ms/82ms, FA=90, FoV=128 mm2, voxel size 2x2x2mm^3, 64 axial slices, one volume with b=0 and 64 volumes with non-collinear directions and diffusion gradient of b=700 s/mm^2.

Image pre-processing

Before any data pre-processing and analysis, all acquisitions were visually inspected by two certified neuroradiologists (MA, CV) and confirmed that they were not affected by critical head motion and that the HC group had no brain lesions. Image pre-processing was performed using BrainCAT [57], a software tool that implements a data processing pipeline tailored for multimodal connectivity analysis using FSL [58], Mricron [59] and Diffusion Toolkit [60] software tools.

The functional acquisition underwent the following pre-processing procedures: removal of the first five volumes (10 seconds) in order to achieve signal stabilization and allow participants to adjust to scanner noise; slice-timing correction in order to compensate for differences in the acquisition of the different slices of each volume; motion correction; removal of non-brains structures; non-linear normalization to Montreal Neurological Institute (MNI) standard space; spatial smoothing using an 8 mm Full-With at Half-Maximum (FWHM) Gaussian filter; band-pass temporal filtering at 0.01-0.08 Hz. No participant exceeded the motion limits set at 2mm in translation and 2 mm in rotation.

The diffusion-weighted data was also processed with BrainCAT. The preprocessing procedures included the correction for eddy current distortions and head movements

followed by the removal of non-brain tissues. Additionally, the gradients matrix was rotated according to the spatial transformations applied during the distortions and movement correction step. Then, the tensor model was fitted to each voxel using the rotated gradients, in which several scalar indices are calculated, including fractional anisotropy, mean diffusivity, axial diffusivity and radial diffusivity maps. Whole-brain tractography was performed using the "Streamline" algorithm distributed with the Diffusion Toolkit with the following parameters: 10 seeds randomly distributed per voxel, step length of 0.2 mm and stopping criteria of minimum FA value of 0.2 or angle change greater than 35º. Finally the clusters extracted from the thresholding procedure applied to each RSN were used in order to filter-in the reconstructed white matter tracts that connect each possible pair of clusters, thus providing (through the streamline count) an estimate of structural connectivity between the regions that form each RSN.

Finally, the identification, segmentation and volumetric quantification of the MS white matter lesions was done manually using the 3D T1 images on the 3D Slicer Software (htts://www.slicer.org/) and was performed by two certified neuroradiologists (MA, CV). Total white mater lesion volume was calculated for each individual that was further introduced as a variable in the regression analyses.

Network construction

Group Independent Component Analysis (gICA) was performed with the entire sample (HC and MS groups) using MELODIC (Multivariate Exploratory Linear Optimized Decomposition into Independent Components) also part of the FSL software [61]. This procedure involves the temporal concatenation of each subject's dataset into a single 4D dataset in order to perform group-wise ICA. The number of components to extract was automatically estimated by the software. RSNs of interest were visually identified. From the 18 components estimated, six components of interest were identified: three components identified as the DMN components, one component identified as the DAN, one component identified as the right VAN and one component identified as the left VAN (see figure 1).

In order to extract the subject's specific components corresponding to the group-wise identified RSNs of interest, the dual regression procedure was employed [62]. This procedure encompasses two stages: (1) linear model fit of the group-wise ICA spatial maps against each subject's functional dataset in order to create matrices describing the temporal dynamics of each component for each subject (spatial regression); (2) linear model fit of each matrix to the corresponding subject functional dataset in order to estimate the spatial correlation maps of each subject (temporal regression). Mean white matter and CSF signals and the six motion parameters resulting from the motion correction step were included in the

matrix for the second step of the dual regression in order to correct for its influence in the subject specific spatial maps. The group spatial map of each network was then thresholded at its 99th percentile in order to extract the clusters that define each network. Afterwards, regions of interest (ROIs) were created as spheres with 5mm radius centered on the peak of each cluster and mean time-series were extracted for each ROI, individually for each subject. For each network, ROI-to-ROI connectivity matrices were built through the Z-transformed Pearson correlation coefficients between each possible pair of ROI time-series computation.

Finally, for the whole-brain connectivity analysis, mean time-series for 116 regions from the Anatomical Automatic Labeling (AAL) atlas were extracted and, similarly to the RSNs analysis, connectivity matrices were built through Z-transformed Pearson correlation coefficients between the 116 time-series.

Statistical Analysis

Results were analyzed in the IBM SPSS Statistics software, v.22 (IBM, New York) and with Matlab. Comparisons between groups were performed with two-tailed independent-samples t-test. For all these comparisons, the significance level was set at 0.05. Values are presented as mean ± standard deviation of the mean.

Voxel-wise between-group differences in RSNs FC maps were performed with *randomise*, a tool distributed with FSL, which implements a permutation-based procedure for statistical inference. For each RSN, independent-samples t-tests were performed using the subject specific RSN maps as inputs. 10000 random permutations were used and results were considered significant at $p < 0.05$ with family-wise error (FWE) correction for multiple comparisons, using the threshold-free cluster enhancement (TFCE) procedure that enables the detection of widespread differences.

Regarding intra-network connectivity of each RSN, group comparisons were performed with independent samples t-tests using each ROI-to-ROI z-transformed correlation coefficient as the dependent variable. Afterwards, multiple regressions were performed within the MS group in order to test for associations between functional connectivity and MS lesion volume, months after diagnosis and the cognitive efficiency composite (calculated considering the average of both SDMT and PASAT z-scores, described above). These three variables were all included in the same model in order to assess the effect of one variable while controlling for the others. Age was also included as covariate in the multiple regression models. Results were considered significant at $p < 0.05$, as reported elsewhere [45].

Whole brain connectivity networks were analyzed with a network inference procedure named Network Based Statistic (NBS) [63]. This procedure consisted, firstly, in the application of a statistical model independently for each connection. Then, a primary threshold (e.g. p <

0.005) is applied and the size of the sub-networks for med by connected supra-threshold connections are tested against the null hypothesis, specifically that a network of similar size would be formed by chance using a permutation procedure, after the application of a similar primary threshold. In the present study, a primary threshold of $p < 0.005$ was used and 5000 permutations were performed. Results were considered significant at $p < 0.05$ corrected for multiple comparisons at the network level. Similarly to intra-network connectivity, independent samples t-tests were used to test for group differences.

Results

Demographic and cognitive characteristics of the participants

Groups did not differ in terms of age [$t(42)= -0.87$, $p>.05$], gender [$\chi^2(1)= 0.16$, $p>.05$] or handedness proportions [$\chi^2(2)= 3.53$, $p>.05$]. In terms of cognitive performance, groups differed in SDMT [$t(42)=4.68$, $p<.001$] and PASAT [$t(42)=2.68$, $p<.05$] outcomes, with the MS group displaying lower scores in both measures. Likewise, groups also differed in the Cognitive Efficiency Composite [$t(42)= 4.26$, $p<.001$], with the MS group displaying lower z-scores (worse performance) – see table 1.

Table 1 – Demographic and cognitive characteristics of participants from HC and MS groups

	HC (n=21) M (SD)	MS (n=23) M (SD)	Differences between groups *P* values
Gender (F/M)	14/7	14/9	0.690 [a]
Mean age (years)	35.95 (7.27)	38.17 (9.42)	0.390 [b]
Handedness	19 right-handed; 2 left-handed; 1 ambidextrous	23 right-handed	0.172 [a]
SDMT	61.43 (11.70)	45.35 (11.08)	<0.001 [b]
PASAT	43.86 (10.45)	35.22 (10.88)	0.01 [b]
Cognitive Efficiency Composite	0.03 (0.91)	-1.09 (0.85)	<0.001 [b]

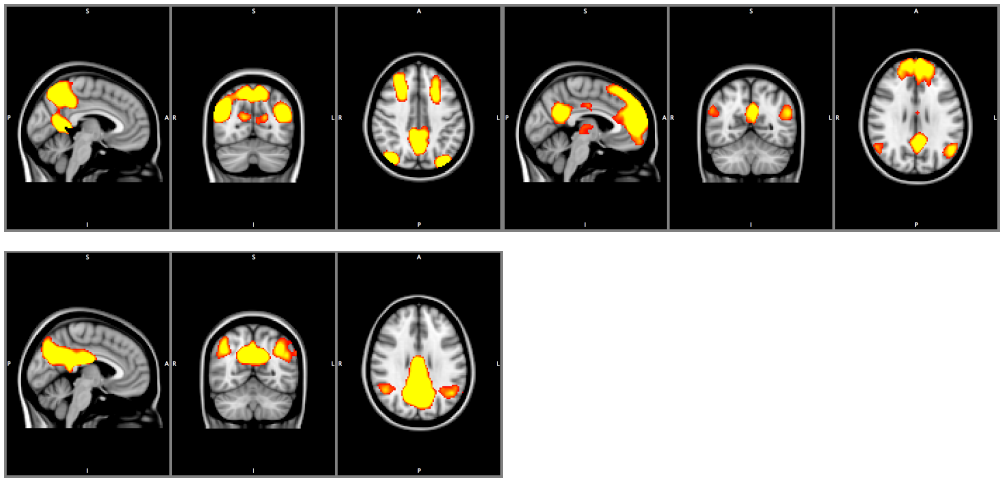
[a]Chi-square test; [b]Unpaired t test.

Resting State Networks Functional and Structural Connectivity

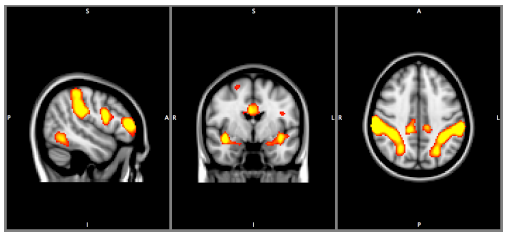
The DMN was identified in the resting state conditions at the whole group level (MS and HC), and four main components were extracted from three different maps, namely the

posterior cingulate cortex and adjacent precuneus; the medial prefrontal cortex; the bilateral inferior parietal cortex; and the left inferior temporal cortex [64] - (see figure 1A). In the DAN, the main components included the bilateral inferior parietal lobule, the bilateral inferior frontal gyrus, the anterior cingulate, bilateral insula and the medial occipital gyrus (see figure 1B). Finally, a right lateralized VAN was identified with major clusters being identified in the right middle and superior frontal gyrus, the right parietal lobe, the left superior parietal lobe, the left inferior frontal gyrus and bilateral cerebellum (see figure 1C). Group statistics of the DMN, DAN and VAN are described in a supplemental table. No group differences were observed in the functional connectivity measures of the DMN, DAN and VAN when using a voxel-wise connectivity approach. Regarding structural connectivity, the results derived from the combination of the clusters extracted from the RSNs and the whole-brain tractography did not displayed any white matter tracts connecting the subregions of the DMN, VAN and DAN.

A)



B)



C)

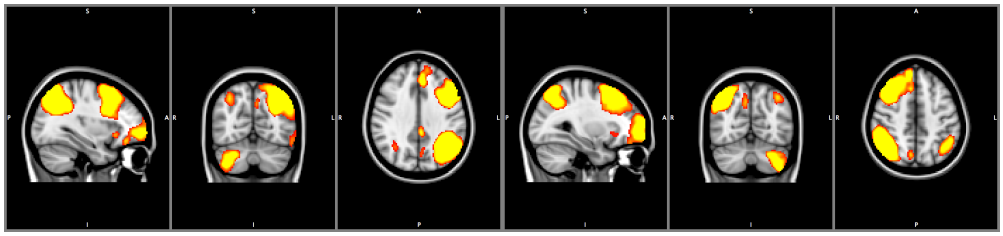


Figure 1 – Group (MS and HC) Functional Connectivity Map of the DMN, DAN and VAN: A) DMN, B) DAN, C) Left VAN in the right panel and Right VAN in the left panel.

Default Mode Network Connectivity Matrices

Differential mean functional connectivity coefficients between the DMN subregions are illustrated in Figure 2. We observed decreased functional connectivity between specific paired subregions of the DMN in the MS group when compared with HC ($p<0.05$) in the following paired regions: right middle frontal gyrus and right occipital cortex ($T=2.15$, $p=0.04$), right fusiforme gyrus and right middle occipital gyrus ($T=2.09$, $p= 0.04$) and between the left precuneus and left angular gyrus ($T=2.59$, $p= 0.01$) – see figure 2.

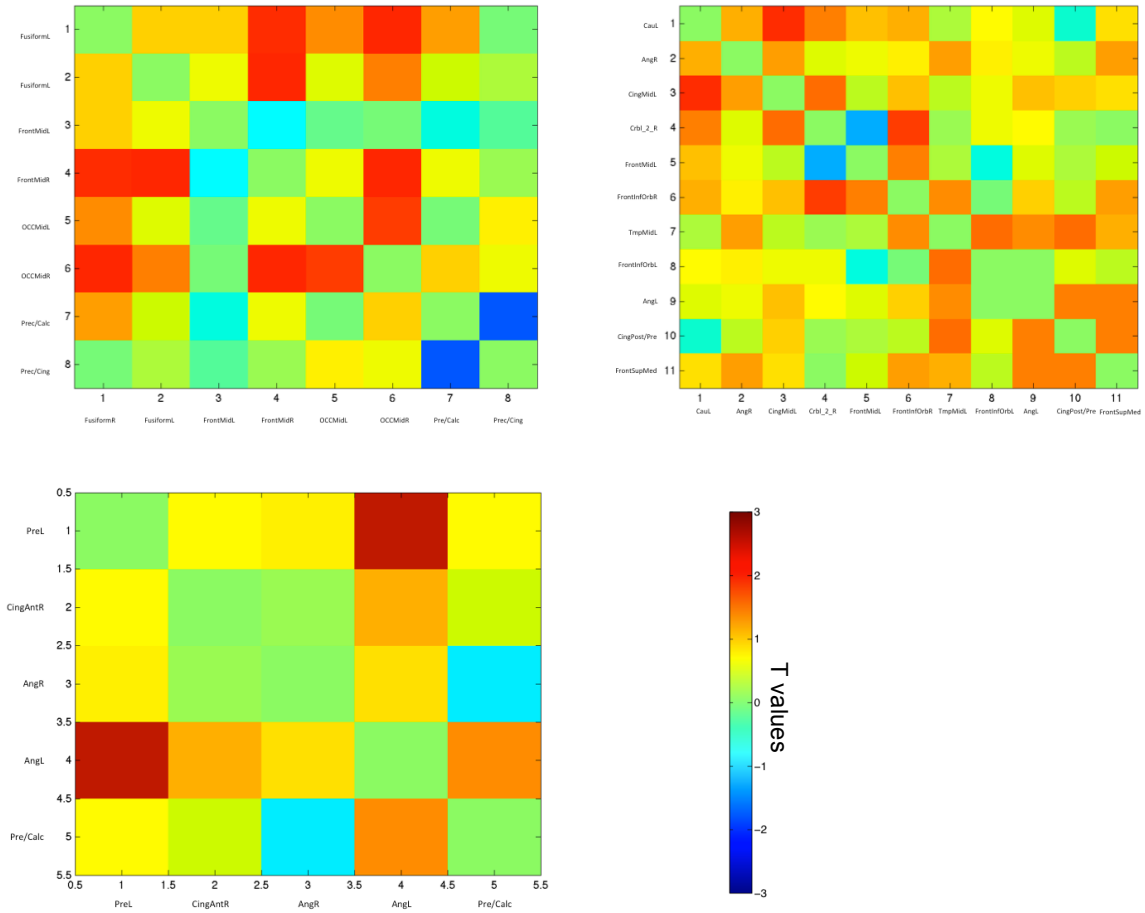


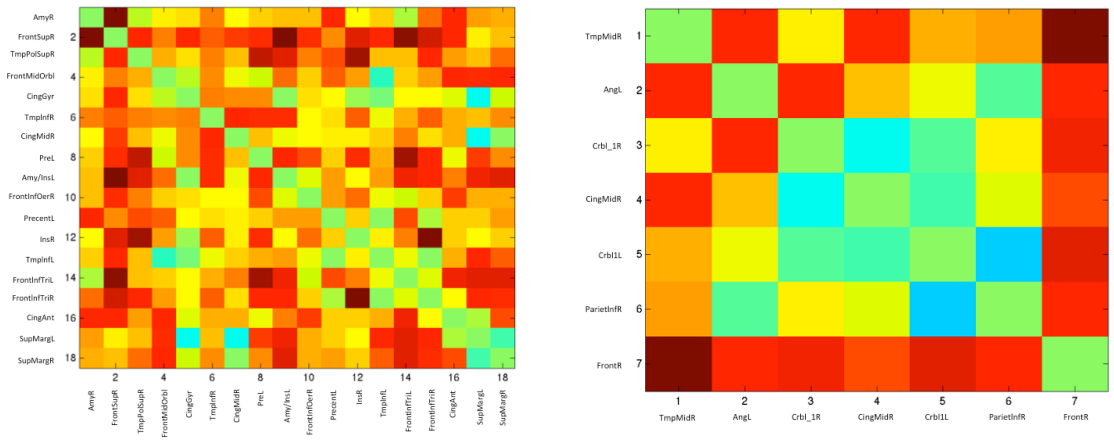
Figure 2 – Correlation Matrix of Functional Connectivity within the Subregions of the DMN; the color bar indicates the magnitude of the group differences (HC > MS are represented by hot colors whereas HC < MS are represented by cold colors); brain subregions are described in supplemental data.

Dorsal and Ventral Attention Networks

The functional connectivity strength in specific edges of the DAN was decreased in MS when compared with the HC; particularly between the right superior frontal gyrus and several subregions: the right amygdala ($T=3.06$, $p=0.003$), the superior temporal gyrus ($T=2.08$, $p=0.04$), the inferior temporal gyrus ($T=2.03$, $p=0.006$), the cingulate gyrus ($T=2.09$, $p=0.04$), the left insula (left: $T=3.26$, $p=0.002$). Additional paired subregions showed lesser functional

connectivity in MS, namely between the right amygdala and the left precentral gyrus (T=2.12, p=0.004), right amygdala and anterior cingulate (T=2.19, p=0.03), and between the superior temporal gyrus and the left precuneus (T=2,88, p=0,02), when compared with the HC – see figure 3A. Regarding the VAN, a decreased functional connectivity was found between the pair left medial superior frontal gyrus and the right cerebellum (T=2.68, p=0.01), and between the right middle temporal gyrus and the left angular gyrus (T=2.20, p= 0.03) and the right cingulate (T=2.12, p=0.04) in the clinical group. Additionally, lesser functional connectivity in the right frontal pole and the following regions: left angular gyrus (T=2.04, p=0.04), right middle temporal gyrus (T=2.91, p=0.006), bilateral cerebellum (right T=2.32, p=0.03, left: T=2.35, p=0.02) was also observed in the MS group, when compared with HC. Finally, the strength of connectivity between the right cerebellum and left angular gyrus was also decreased in MS (T=-2,08, p=0,04) – see figure 3B and 3C.

A) B)



C)

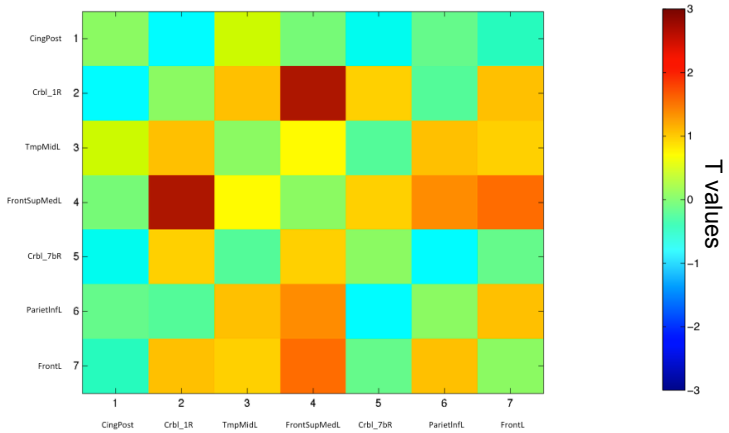


Figure 3 - Correlation Matrix of Functional Connectivity within the Subregions of the A) DAN; B) Right VAN; C) Left VAN; the color bar indicates the magnitude of the group differences (HC > MS are represented by hot colors where as HC > MS are represented by blue colors.

Whole Brain Connectivity

When we tested the connectivity at the whole brain network level, a sub-network of reduced connectivity was found in the MS group, using a primary threshold level of $p \leq 0.005$, with a network-level significance of $p = 0.0428$. The affected network extends from anterior to posterior areas in both hemispheres, and comprised 49 different nodes and 134 connections. The key regions in this network, identified as having five or more affected connections within the affected sub-network, are the bilateral rolandic operculum, the left supplementary motor area, the right Heschl area, the left cerebellum and the right cerebellum crus. No other significant networks were found. Moreover, regarding the distance among network nodes, both short range and long-distance connectivity links were affected. The brain networks were visualized using BrainNet viewer see figure 4.

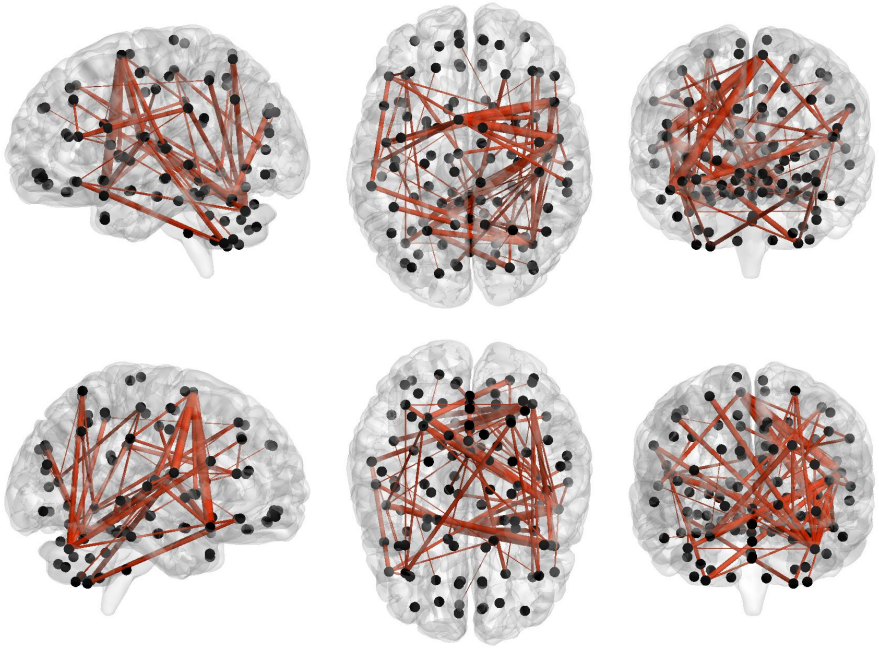


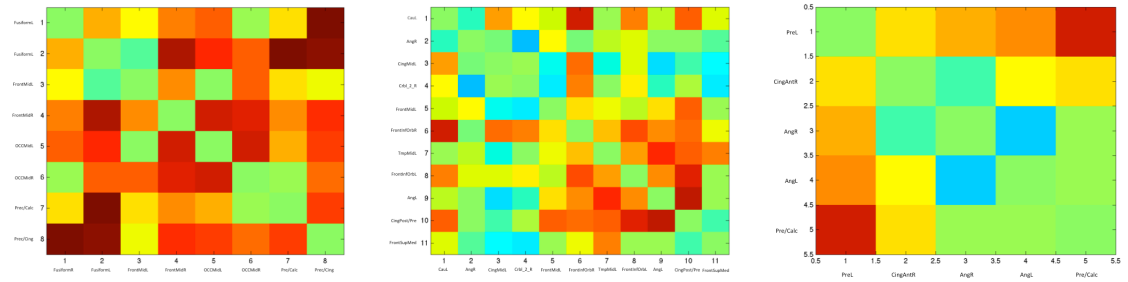
Figure 4: Whole brain functional connectome.

Analysis of Functional Connectivity, Clinical and Cognitive Efficiency in MS group

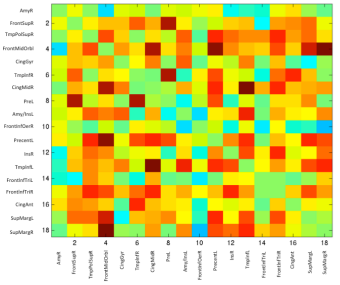
We performed a regression analysis in order to analyze the strength of the connection between the subregions of the DMN, DAN and VAN, disease duration and cognitive efficiency measures in the MS group. Results showed that increased disease duration was

associated with increased functional connectivity in several regions of the DMN, DAN and VAN (see Figure 5).

A)



B)



C)

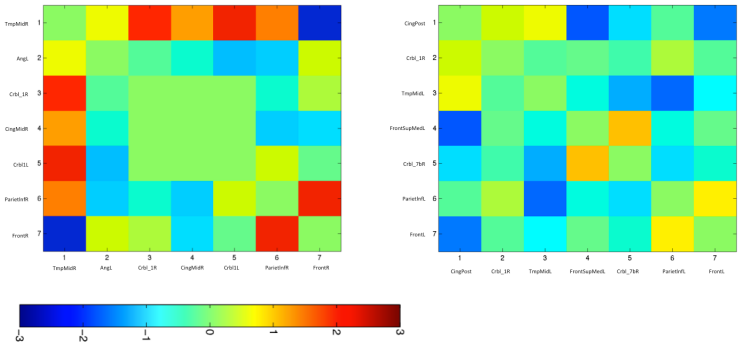
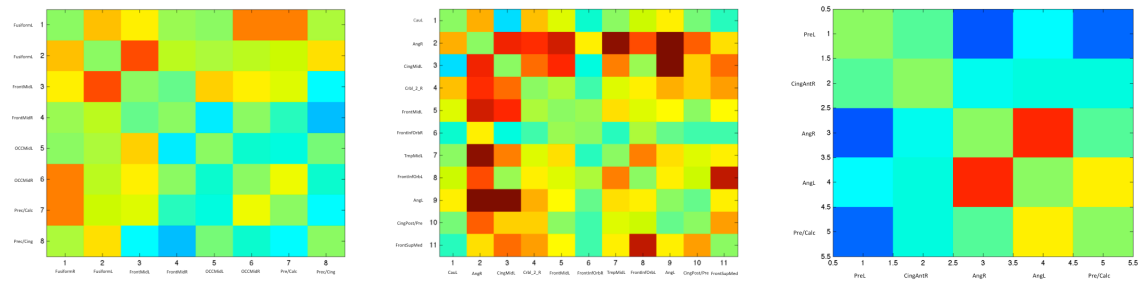


Figure 5 – Functional Connectivity Correlation Matrix between subregions of the DMN (A), DAN (B) and right and left VAN respectively (C) and disease duration in MS; hot colors represented positive correlations whereas cold colors represent negative correlations

Finally, the cognitive efficiency composite was positively associated with several paired frontal regions of the DMN and DAN (see Figure 6).

A)



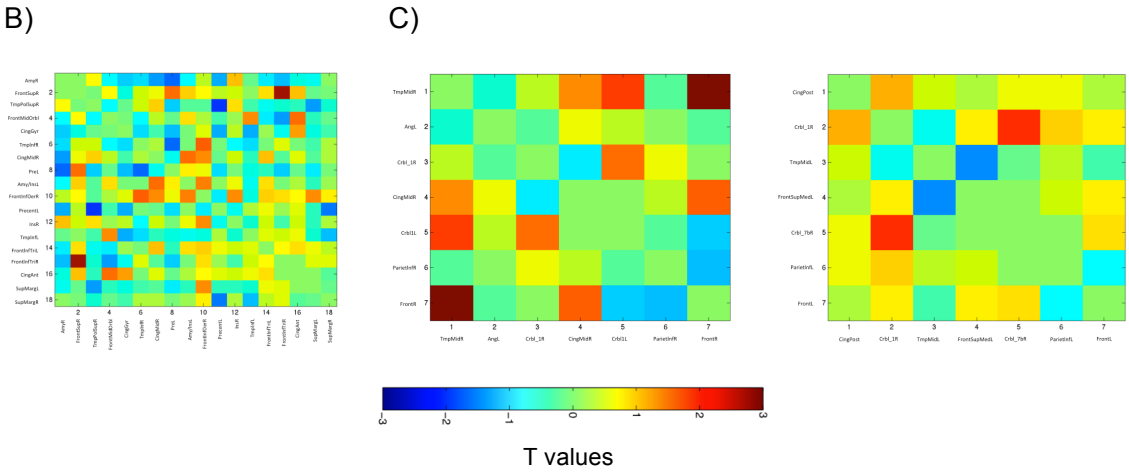


Figure 6 – Functional Connectivity Correlation Matrix between subregions of the DMN (A), DAN (B) and right and left VAN, respectively (C) and cognitive efficiency composite; hot colors represented positive correlations whereas cold colors represent negative correlations.

Discussion

In this study we studied the functional connectivity using a whole brain network level and with a regional approach of the DMN, DAN and VAN in MS and in a healthy control group. Additionally, within the MS group, we also analyzed the impact of disease duration and cognitive efficiency in the strength of functional connectivity within the subregions of the DMN, DAN and VAN. Our results showed no group differences when we used a voxel-wise connectivity approach (the group analysis did not survive to the multiple comparisons correction). However, when we employed a regional ROI-to-ROI connectivity analysis, we observed a decreased functional connectivity in MS in specific inter-regional paired subregions of the DMN, DAN and VAN. Specifically, differences were observed mainly in the strength of connectivity between subregions of the dorsal and ventral attention networks, involving the frontal and parietal cortex (right frontal pole, right superior frontal gyrus, left medial frontal gyrus, cingulate gyrus, left angular gyrus). Regions of the cerebellum and temporal lobe also showed reduced functional connectivity in the clinical group. These results are consistent with studies showing lesser degree of functional connectivity in regions of the attentional networks both in patients with RRMS with cognitive impairment [41,65,66] and cognitively preserved [65], which are also evident in pediatric samples [67]. This decreased functional connectivity in the attentional networks is also consistent with behavioral data derived from the current study but also with other evidence showing that impairments in attention and information processing speed are a distinctive feature of MS that may contribute to impairments in other cognitive functions [66,68]. Finally, these results are also

consistent with our whole brain functional connectome, in which we found a global reduced network connectivity comprising both hemispheres, including both short-range and long-distance connectivity nodes, being consistent with previous data [67]. Specifically, the most affected networks included the dorsal prefrontal and temporal regions and the left cerebellum, brain areas that are interconnected and known to be involved in information processing, attention, working memory and other cognitive tasks in MS [69,70] but also in healthy adults [71].

Although the main result found for MS was a global decreased functional connectivity in several paired subregions of the DMN, DAN and VAN, we also observed that increased disease duration was associated with an increase in the strength of the connections within these RSNs. These results are consistent with a brain reorganization or adaptive cerebral mechanism hypothesis that take place as a result of brain inflammation and as the lesion load progresses [72]. However, some studies propose that increased resting state functional connectivity is only observable in early stages of the disease [32] with this hyperconnectivity being further replaced by an opposite pattern of reduced functional connectivity during disease progression. Our study does not allow us to assess the change in the patterns of functional connectivity in the RSNs, as we have incorporated patients with MS with a wide range of disease duration (from 1 to 228 months) in a transversal study. Nevertheless, we controlled in the regression model for the effects of white matter lesion volume, age, and cognitive functioning, a methodological approach that has not been considered by the majority of studies. Also, this compensatory mechanism is not consistent with evidence showing that increased functional connectivity of the DMN and control networks is associated with a reduced anatomical connectivity and decline in cognitive efficiency in early MS [37].

In fact, other hypothesis have been emerged [73], as cognitive dysfunction has been related with both decreased [41,65] and increased DMN functional connectivity [33,45,74]. In fact, there are limited studies that tried to establish the link between resting-state functional connectivity and cognition in MS, with the results being contradictory [33,37,41,45,65,74]. Here, we found that increased functional connectivity of several pairs of the DMN and DAN, particularly interconnecting with the frontal regions, were associated with an increased cognitive efficiency. This positive association between functional connectivity and cognitive efficiency is consistent with indirect evidence derived from fMRI studies investigating memory, attention and executive processes. Specifically, an enhanced recruitment of several cortical and subcortical regions and a more widespread brain activation on task-specific regions has been consistently described in MS, which were interpreted as a neural compensatory mechanism [55,75]. Moreover, this enhanced recruitment – neuroplasticity

hypothesis – is increased with the cognitive load and across ages, stages and disease progression [76], which may still be observed despite extensive pathology [77].

Although we observed regional reduced functional connectivity in MS, these differences were not observed when we used a voxel-wise analysis, which is inconsistent with previous studies [34,35,37,41]. These differences may be related with the methods used to extract the group RSN maps and the use of stringent statistical methods to control for multiple comparisons as we used in the current study. Some methodological differences include the extraction of functional connectivity maps using *a priori* defined masks with specific regions of interest, as seed regions, and the use uncorrected α-levels. Additionally, in order to assess the RSN structural connectivity, we combined the clusters extracted from the DMN, DAN and VAN and the whole-brain tractography. However, we did not observe any result due to a methodological limitation; namely, in several pairs of clusters we did not have any interconnecting streamline, and, more importantly, for the same pair of clusters, the analysis did not accomplish to reconstruct streamlines for every subject, even when an existing tract is known to exist. This is possibly due to subtle misplacements of the clusters relative to the DTI data or to the quality of the DTI data. In order to overcome this issue, future analysis should try to use structural connectivity estimates derived from probabilistic tractography rather than deterministic tractography, since they provide an estimate for the likelihood of the existence of white matter tract, which is a defined value for every subject. Additionally, some of these methods are known compensate for the issue of crossing fibers, a well-known limitation of DTI [78]. Other limitation of this study is due to the heterogeneity of patients studied in terms of disease course and duration. Future studies should address the longitudinal association between cognitive efficiency and MS in early and chronic MS. Also, the widespread impact of lesions in white matter and in gray matter structures serving RSN should be assess, together with cognitive functioning.

Conclusions

MS is an inflammatory, demyelinating disease of the central nervous system that affects both gray matter and white matter. In this study we assessed the functional connectivity in MS at a regional and whole brain level. Overall, we found a global hypoconnectivity pattern in MS, when compared with the HC. Despite this global decreased functional connectivity, we observed that increased connectivity in specific subregions of the DMN, DAN, VAN were associated with disease duration and cognitive efficiency, after controlling for age and total white matter lesion volume. This regional and whole brain level

approach is useful to study MS and can provide new insights regarding the brain functional reorganization and compensatory mechanisms that occur within the course of the disease.

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UNIVERSIDADE DO PORTO

STRUCTURAL AND FUNCTIONAL CONNECTIVITY IN MULTIPLE SCLEROSIS

Supplemental Data

Table I – DMN Cluster Statistics

Cluster Index	Voxels	Value	x	y	z	AAL Label
8	2375	12.1	-4	-62	50	Left Precuneus
8		10.9	6	-64	52	Right Precuneus
8		8.41	-4	-44	42	Left Cingulate Gyrus
8		7.88	6	-40	42	Right Cingulate Gyrus
7	2189	14.3	14	-56	14	Right Posterior Cingulate
7		12.6	-10	-56	8	Left Posterior Cingulate
6	1542	14.1	40	-74	32	Right Inferior Parietal Lobe – angular gyrus
5	1155	14.4	-36	-80	32	Left Inferior Parietal Lobe – angular gyrus
4	922	9.87	26	26	42	Right Middle Frontal Gyrus
3	510	7.85	-22	10	48	Left Middle Frontal Gyrus
3		7.24	-24	20	44	Left Middle Frontal Gyrus
3		6.11	-22	28	34	Left Middle Frontal Gyrus
2	205	8.72	-28	-38	-18	Left Fusiform Gyrus
1	129	7.08	24	-34	-18	Right Parahippocampal Gyrus
1		7.06	30	-36	-16	Right Fusiform Gyrus

Threshold. T=5.388

Cluster Index	Voxels	Value	x	y	z	AAL Label
11	6861	10.3	-4	56	14	Left Medial Frontal Gyrus
11		8.22	-6	42	46	Left Medial Frontal Gyrus
11		7.95	6	56	10	Right Medial Frontal Gyrus
11		7.54	-6	50	38	Left Superior Frontal Gyrus
11		7.15	-18	52	26	Left Superior Frontal Gyrus
11		6.92	10	58	28	Right Superior Frontal Gyrus
10	920	8.21	-4	-52	26	Left Cingulate Gyrus
10		6.59	4	-52	24	Right Precuneus
9	397	5.86	-48	-66	32	Left Parietal Lobe – Angular Gyrus
8	296	6.03	-30	18	-22	Left Inferior Frontal Gyrus
8		5.19	-40	30	-18	Left Inferior Frontal Gyrus
7	214	5.15	-60	-10	-26	Left Inferior Temporal Gyrus
7		5.14	-60	-16	-18	Left Middle Temporal Gyrus
6	172	5.13	34	24	-22	Frontal Inferior Frontal Gyrus
6		4.74	46	30	-16	Right Inferior Frontal Gyrus
5	73	4.88	-40	18	44	Left Middle Frontal Gyrus
4	35	4.16	26	-86	-36	Right Cerebellum Posterior Lobe
4		4.08	30	-84	-38	Right Cerebellum Posterior Lobe
3	22	4.71	-2	-16	36	Left Cingulate Gyrus
2	20	4.24	56	-58	28	Right Angular Gyrus
1	16	4.33	-12	10	8	Left Caudate Nucleus

Threshold. T=3.929

Cluster Index	Voxels	Value	x	y	z	AAL Label
5	6918	13.8	10	-64	32	Right Precuneus
5		12.6	4	-22	26	Right Precuneus
5		12.4	-6	-70	32	Left Precuneus
5		12.3	0	-68	32	Left Precuneus
5		10.5	2	-50	30	Right Posterior Cingulate
5		9.61	-6	-40	22	Left Posterior Cingulate
4	1039	6.45	-38	-60	42	Left Inferior Parietal Lobule – Angular Gyrus
4		5.01	-46	-60	30	Left Parietal Lobe - Angular Gyrus
4		4.24	-52	-52	20	Left Middle Temporal Gyrus
4		3.62	-60	-60	34	Left Supramarginal Gyrus
3	896	6.4	44	-58	38	Right Angular Gyrus
2	139	4.35	4	34	14	Right Anterior Cingulate
2		4.3	2	42	6	Right Anterior Cingulate
1	35	4.28	0	-52	64	Left Precuneus

Threshold. T=3.426

Table II – DAN Cluster Statistics

Cluster Index	Voxels	Value	x	y	z	AAL Label
18	3182	10.7	62	-30	30	Right Supramarginal Gyrus
18		6.65	38	-40	40	Right Inferior Parietal Lobule
18		6.04	8	-60	60	Right Precuneus
18		5.76	26	-62	42	Right Superior Occipital Gyrus
18		5.33	40	-42	58	Right Postcentral Gyrus
18		4.46	22	-46	68	Right Superior Parietal Gyrus
17	2109	10.6	-60	-30	30	Left Supramarginal Gyrus
17		5.63	-24	-64	42	Left Superior Parietal Gyrus
16	517	6.51	-2	12	26	Left Anterior Cingulate
16		6.02	4	6	30	Right Cingulate – mid region
15	429	6.82	48	40	10	Right Inferior Frontal Gyrus – pars triangularis
14	372	6.42	-46	40	8	Right Inferior Frontal Gyrus – pars triangularis
14		5.33	-36	36	12	Left Right Inferior Frontal Gyrus – pars triangularis
13	343	6.77	-52	-62	-8	Left Inferior Temporal Gyrus
12	342	6.87	40	-8	-8	Right Insula
11	340	6.09	-46	4	22	Left Precentral Gyrus
10	338	6.18	50	6	22	Right Inferior Frontal – pars opercularis
9	338	6.89	-38	-14	-8	Left Insula
9		5.56	-38	0	-10	Left Insula
9		4.55	-26	0	-20	Left Amygdala
9		4.5	-22	-2	-18	Left Amygdala
8	228	5.55	-10	-56	62	Left Precuneus
7	191	5.96	14	-34	38	Right Cingulare Gyrus – mid region
6	174	5.54	54	-58	-8	Right Inferior Temporal Gyrus

5	70	5.3	-12	-30	36	Left Cingulate Gyrus
4	27	4.55	-28	36	-18	Left Middle Frontal – pars orbicularis
3	12	4.46	60	8	-2	Right Superior Temporal Gyrus
2	10	4.47	22	0	62	Right Superior Frontal Gyrus
1	5	4.49	22	0	-20	Right Amygdala

Table III – VAN Cluster Statistics

Left VAN

Cluster Index	Voxels	Value	x	y	z	AAL Label
7	4694	12.1	-44	10	28	Left Inferior Frontal Gyrus – pars opercularis
7		10	-44	50	-6	Left Middle Frontal Gyrus
7		9.88	-48	24	22	Left Inferior Frontal Gyrus – pars triangularis
7		8.66	-32	14	50	Left Middle Frontal Gyrus
6	3643	13.3	-34	-64	44	Left Inferior Parietal Lobule
6		10.7	-44	-52	48	Left Inferior Parietal Lobule
6		10.5	-42	-48	40	Left Inferior Parietal Lobule
5	237	7.22	36	-70	-48	Right Cerebellum - Crus
5		5.97	30	-66	-36	Right Cerebellum - Crus
4	182	7.5	-4	34	38	Left Superior Frontal Gyrus – medial region
4		7.25	-6	26	42	Left Superior Frontal Gyrus – medial region
3	137	6.93	-64	-44	-8	Left Middle Temporal Gyrus
3		5.99	-54	-58	-18	Left Inferior Temporal Gyrus
2	118	7.96	12	-78	-28	Right Cerebellum - Crus
1	16	6.2	-4	-38	30	Left Cingulate Gyrus - posterior section

Threshold. T=5.560

Right VAN

Cluster Index	Voxels	Value	x	y	z	AAL Label
7	5497	13.8	44	24	38	Right Middle Frontal Gyrus
7		10.8	40	60	0	Right Middle Frontal Gyrus
7		9.81	36	16	54	Right Superior Frontal Gyrus - mid region
7		9.17	36	10	50	Right Middle Frontal Gyrus – mid region
7		8.36	46	52	-8	Right Middle Frontal Gyrus – mid region
7		8.07	24	30	50	Right Superior Frontal Gyrus
6	2993	15.6	46	-52	48	Right Inferior Parietal Lobule
6		15.3	40	-60	50	Right Angular Gyrus
5	404	8.57	-38	-70	-48	Left Cerebellum Posterior Lobe - Crus
5		6.43	-30	-66	-36	Left Cerebellum Posterior Lobe - Crus
5		5.77	-36	-72	-32	Left Cerebellum - Uvula
4	49	6.79	4	-30	34	Right Cingulate Gyrus – mid region
3	45	6.15	-10	-78	-28	Left Cerebellum Posterior - Crus
2	26	5.87	-40	-60	48	Left Angular Gyrus
1	12	5.87	66	-28	-12	Right Middle Temporal Gyrus

Threshold. T= 5.518

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